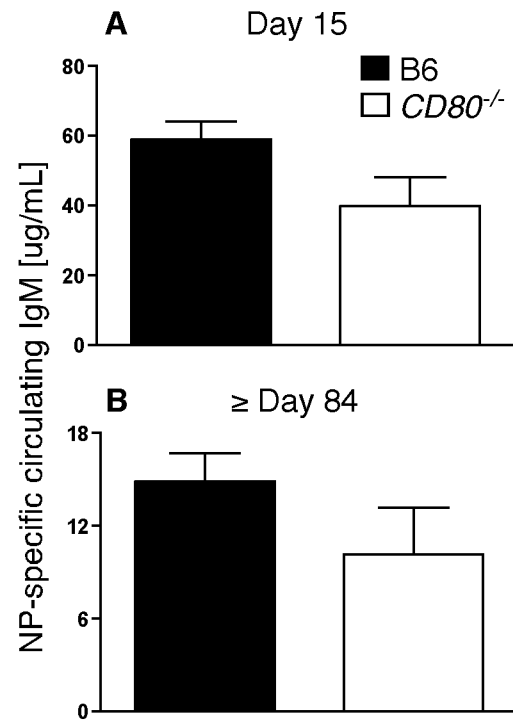
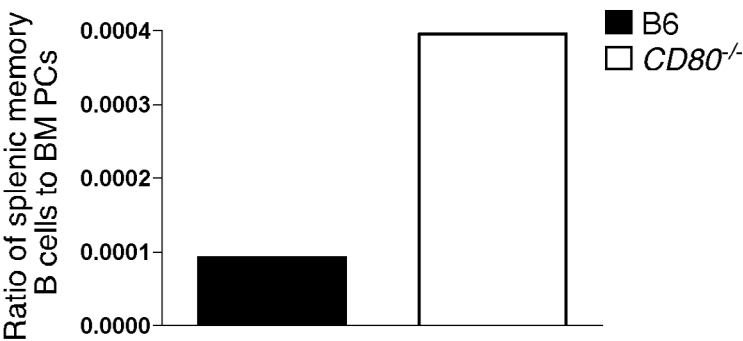


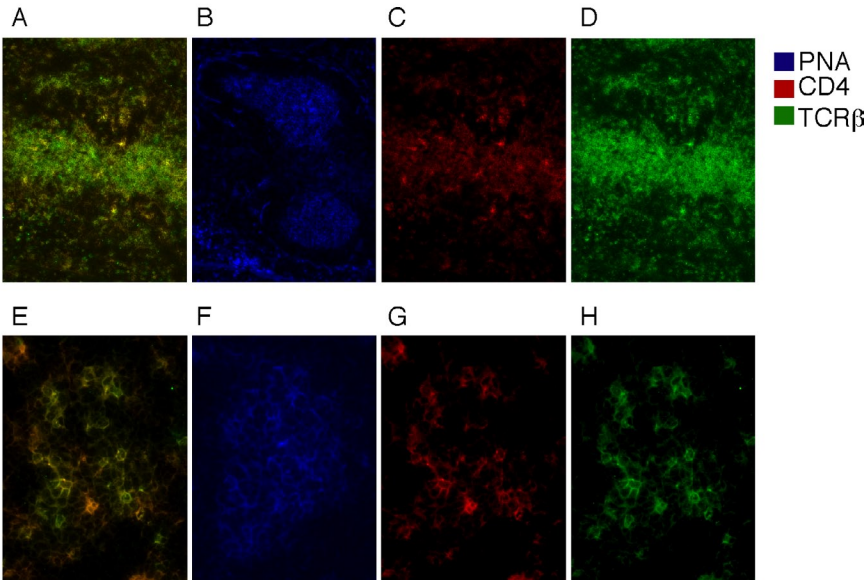
Supplemental Figure 1



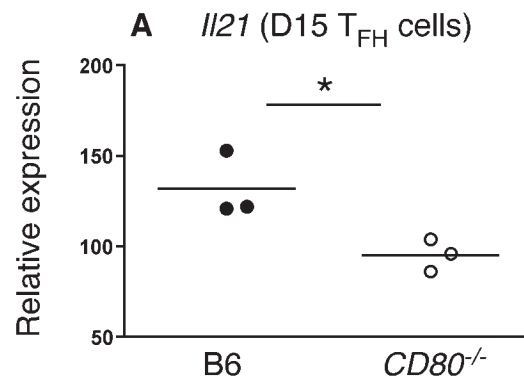
Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4



## Supplemental Figure Legends

### **Supplemental Figure 1: IgM circulating Ab in CD80-deficient mice is comparable to WT mice.**

NP<sup>+</sup>IgM<sup>+</sup> circulating Ab in B6 mice (black bars) and *CD80*<sup>-/-</sup> mice (white bars) was assessed by ELISA at day 15 (**A**; n=6 (B6), n=7 (*CD80*<sup>-/-</sup>)) and ≥ day 84 (**B**; n=2 (B6), n=3 (*CD80*<sup>-/-</sup>)) post-immunization with NP-CGG in alum.

### **Supplemental Figure 2: The ratio of memory B cells to BM PCs is greater in CD80-deficient mice compared to WT.**

Ratio of splenic memory B cells to BM PCs in B6 mice (black bars) and *CD80*<sup>-/-</sup> mice (white bars) present at least 12 weeks post-immunization with NP-CGG in alum.

**Supplemental Figure 3.** CD4 and TCRβ co-stain T cells within the GC. *CD80*<sup>-/-</sup> mice and their B6 controls were immunized and at multiple time-points post-immunization spleens were frozen. Frozen spleen sections were stained with antibodies to CD4, PNA and TCRβ. A series of tiled images were obtained at 20x and merged into a composite. (**A-D**) and (**E-H**) show two representative areas from a B6 mouse from day 15 post-immunization. **A-D** is a lower power view that shows two PNA<sup>+</sup> GC on either side of a T cell area, which provides a positive control region for both CD4 and TCRβ staining. The staining of both CD4 and TCRβ are readily visible in both GC. **E-H** is magnified view (approximately 4-fold enlarged from **A-D**) of a different GC to allow visualization of individual cells. **A** and **E** are overlays of (**C-D**) and (**E-H**) respectively,

to most clearly show the co-staining of CD4 and TCR $\beta$ . (**B, F**) is PNA in blue; (**C, G**) is CD4 in red; and (**D, H**) is TCR $\beta$  in green.

**Supplemental Figure 4.** *CD80*<sup>-/-</sup> (open circles) and B6 (closed circles) mice were immunized with NP-CGG in alum and splenocytes sorted for CD4 expression as well as ICOS expression and CCR7 downregulation in conjunction with CXCR5 expression. *Il21* mRNA expression was assessed by qPCR. Data are shown as fold change as calculated by  $2^{(\text{actin Ct} - \text{cytokine Ct})}$ . Each data point is derived from 3 separate mice; spleens were combined before sorting. \**P* < 0.05 as determined by a two-tailed Student's t test.